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(71) Applicant: MASSACHUSETTS INSTITUTE OF TECHNOLOGY [US/US]; 77 Massachusetts Avenue, Cambridge, MA 02139 (US).

(72) Inventors: OKADA, Junichi; 1642-212 Kamariyacho, Kanazawaku, Yokohama (JP). COHEN, Smadar; 12 Tfuzoth Israel Street, 49581 Petah Tikva (IL). LANGER, Robert, S.; 77 Lombard Street, Newton, MA 02185 (US).

(74) Agent: PABST, Patrea, L.; Kilpatrick & Cody, Suite 2800, 1100 Peachtree Street, Atlanta, GE 30309-4530 (US).

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(54) Title: POLYMERIZED LIPOSOMES WITH ENHANCED STABILITY FOR ORAL DELIVERY

(57) Abstract

Pharmaceutical compositions for oral delivery are prepared by encapsulation of compounds to be delivered to the small intestine within polymerized liposomes. The constituent phospholipids and/or the leaflets are polymerized through double bond-containing olefinic and acetylenic phospholipids. Covalently binding the layers through polymerization adds strength, resulting in a less fluid unpolymerized liposome. Polymerized liposomes can also be prepared by chemical oxidation of thiol groups in the phospholipids to disulfide linkages. Biologically active substances, such as a drug or antigen, can be encapsulated during the polymerization by mixing the substances into the liposome components at the time the liposomes are formed. Alternatively, the liposomes can be polymerized first, and the biologically active substance can be added later by resuspending the polymerized liposomes in a solution of a biologically active substance, and sonicating the suspension or by drying the polymerized liposomes to form a film, and hydrating thefilm in a solution of the biologically active substance.

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POLYMERIZED LIPOSOMES WITH ENHANCED STABILITY FOR ORAL DELIVERY

This invention relates to the synthesis, preparation, and use of polymerized liposomes for oral drug and vaccine delivery.

Background of the Invention

Drug delivery takes a variety of forms, depending on the agent to be delivered and the administration route. The most convenient way to administer drugs into the body is by oral administration. However, many drugs, in particular proteins and peptides, are poorly absorbed and unstable during passage through the gastrointestinal (G-I) tract. The administration of these drugs is generally performed through parenteral injection.

Although oral vaccination is more convenient, vaccines are generally given through injection. This is particularly true with killed or peptidic vaccines, because of their low absorbability and instability in the G-I tract. A problem with systemic immunization is that it may not effectively induce mucosal immune responses, particularly production of IgA, that are important as the first defense barrier to invaded microorganisms. For this reason, it would be beneficial to provide oral vaccination, if the problems of low absorbability and instability could be overcome.

Controlled release systems for drug delivery are often designed to administer drugs to specific areas of the body. In the gastrointestinal tract it is important the drug not be eliminated before it has had a chance to exert a localized effect or to pass into the bloodstream.

Enteric coated formulations have been widely used for many years to protect drugs

administered orally, as well as to delay release. Several microsphere formulations have been proposed as a means for oral drug delivery. For example, PCT/US90/06430 and PCT/US90/06433 by Enzytech discloses the use of a hydrophobic protein, such as zein, to form microparticles; U.S. Patent No. 4,976,968 to Steiner, et al. discloses the use of "proteinoids" to form microparticles; and European Patent Application 0,333,523 by The UAB Research Foundation and Southern Research Institute discloses the use of synthetic polymers such polylactic acid-glycolic acid to form microspheres.

Particles less than ten microns in diameter, such as the microparticles of EPA 0,333,523, can be taken up by cells in specialized areas, such as Peyer's patches and other intestinal mucosal lymphoid aggregates, located in the intestine, especially in the ileum, into the lymphatic circulation. Entrapping a drug or antigen in a microparticulate system can protect the drug or antigen from acidic and enzymatic degradation, yet still allow the drug or antigen to be administered orally, where they are taken up by the specialized uptake systems, and release the entrapped material in a sustained manner or are processed by phagocytic cells such as macrophages. When the entrapped material is a drug, elimination of the first-pass effect (metabolism by the liver) is highly advantageous.

Liposomes have been proposed for use as an oral drug delivery system, for example, by Patel and Ryman, FEBS Letters 62(1), 60-63 (1976). Liposomes are typically less than 10 microns in diameter, and, if they were stable to passage through the GI tract, might be absorbed through the Peyer's patches. Liposomes also have some features that should be advantageous for a particulate

system for oral drug or antigen delivery. The phospholipid bilayer membrane of liposomes separates and protects entrapped materials in the inner aqueous core from the outside. Both water-soluble and water-insoluble substances can be entrapped in different compartments, the aqueous core and bilayer membrane, respectively, of the same liposome. Chemical and physical interaction of these substances can be eliminated because the substances are in these different compartments. Further, liposomes are easy to prepare. However, liposomes are physically and chemically unstable, and rapidly leak entrapped material and degrade the vesicle structure. Without fortifying the liposomes, they are not good candidates for oral drug or antigen delivery.

Several methods have been tried to stabilize liposomes. Some methods involved intercalating cholesterol into the bilayer membrane or coating the liposome with polysaccharides. These methods are not useful in making liposome for oral delivery since orally administered liposomes are exposed to an acidic pH in the stomach and bile salts and phospholipases in the intestine. These conditions break down the cholesterol and polysaccharide in the liposomes.

There remains a need for drug and antigen delivery devices that can survive the harsh conditions in the GI tract, and yet effectively deliver the drug and antigen.

It is therefore an object of the invention to provide stable liposomes for use in oral drug and antigen delivery.

It is a further object of the invention to provide methods of preparing stabilized liposomes.

It is still a further object of the invention to provide a method for orally

administering drugs or antigens entrapped within these stabilized liposomes to a patient in need of the drug or antigen.

Summary of the Invention

Pharmaceutical compositions for oral delivery are prepared by encapsulation of compounds to be delivered to the small intestine within polymerized liposomes. The constituent phospholipids and/or the leaflets are polymerized through double bond-containing olefinic and acetylenic phospholipids. Covalently binding the layers through polymerization adds strength, resulting in a less fluid unpolymerized liposome. Polymerized liposomes can also be prepared by chemical oxidation of thiol groups in the phospholipids to disulfide linkages.

Biologically active substances, such as a drug or antigen, can be encapsulated during the polymerization by mixing the substances into the liposome components at the time the liposomes are formed. Alternatively, the liposomes can be polymerized first, and the biologically active substance can be added later by resuspending the polymerized liposomes in a solution of a biologically active substance, and sonicating the suspension. Another method of entrapping a biologically active substance in polymerized liposomes is to dry the polymerized liposomes to form a film, and hydrate the film in a solution of the biologically active substance. The above conditions are typically mild enough to entrap biologically active substances without denaturing them.

As demonstrated by the examples, the less fluid bilayer membrane of the polymerized liposomes suppresses leakage. Further, the detergent-like

bile salts in the intestine cannot extract the phospholipid molecules. These cross-linked membranes are strong enough to maintain their structure even if the phospholipids undergo hydrolysis at low pH and enzymatic degradation by phospholipases. Polymerized liposomes reach the ileum of the GI tract as intact particulates, and are absorbed.

Brief Description of the Figures

Figure 1 is a graph of the percent BSA (Bovine Serum Albumin) released from polymerized liposomes of DODPC (1,2-di(2,4-Octadecadienoyl)-3-phosphatidylcholine), in various solvent systems, as a function of time (in days). The open circles represent release of BSA in a solution buffered to pH 7 with Tris-saline. The darkened triangles represent the release of BSA in a solution of pH 2 saline. The darkened squares represent the release of BSA in a solution of bile (PLA and TCA).

Figure 2 is a graph of the percent BSA (Bovine Serum Albumin) released from unpolymerized liposomes of DODPC (1,2-di(2,4-Octadecadienoyl)-3-phosphatidylcholine), in various solvent systems, as a function of time (in days). The open circles represent release of BSA in a solution buffered to pH7 with Tris-saline. The darkened triangles represent the release of BSA in a solution of pH 2 saline. The darkened squares represent the release of BSA in a solution of bile (PLA and TCA).

Figure 3 is a graph of the percent BSA (Bovine Serum Albumin) released from liposomes of hydrogenated egg phosphatidylcholine and cholesterol, in various solvent systems, as a function of time (in days). The open circles represent release of BSA in a solution buffered to pH 7 with Tris-saline. The darkened triangles

represent the release of BSA in a solution of pH 2 saline. The darkened squares represent the release of BSA in a solution of bile (PLA and TCA).

Figure 4 is a graph of the total absorption of radioactive protein (in picocuries) as a function of time (in hours). The darkened bars represent the absorption of protein from polymerized liposomes. The hashed bars represent the absorption of protein from unpolymerized liposomes. The white bars represent the absorption of protein from a protein solution.

Detailed Description of the Invention

The polymerized liposomes, methods of preparing these liposomes, and methods of using these polymerized liposomes for oral delivery are described in more detail below.

Preparation of Polymerized Liposomes

As used herein, the term "liposome" is defined as an aqueous compartment enclosed by a lipid bilayer. (Stryer, Biochemistry, 2d Edition, W.H. Freeman & Co., p. 213 (1981). The liposomes can be prepared by a thin film hydration technique followed by a few freeze-thaw cycles. Liposomal suspensions can also be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811, incorporated herein by reference in its entirety.

As used herein, the term "polymerized liposome" is defined as a liposome in which the constituent phospholipids are covalently bonded to each other. The phospholipids can be bound together within a single layer of the phospholipid bilayer (the leaflets) and/or bound together between the two layers of the bilayer.

The degree of crosslinking in the polymerized liposomes can range from 30 to 100

percent, i.e., up to 100 percent of the potential bonds are made. The size range of the polymerized liposomes is between approximately 15 nm to 10 μm. The polymerized liposomes can be loaded with up to 100% of the material to be delivered, when the material is hydrophobic and attracted by the phospholipid layers. In general, five to 40 percent of the material is encapsulated when the material is hydrophilic.

As used herein, the term "trap ratio" is defined as the ratio of inner aqueous phase volume to total aqueous phase volume used.

As used herein, the term "radical initiator" is defined as a chemical which initiates free-radical polymerization.

As used herein, the term "reverse phase evaporation technique" is defined as a method involving dissolving a lipid in an organic solvent, adding a buffer solution, and evaporating the organic solvent at reduced pressure, as described by Skoza, F. Jr., and Papahadjopoulos, D., Proc. Natl. Acad. Sci. USA, Volume 75, No. 9, pp. 4194-4198 (1978).

As used herein, the term "freeze-thaw technique," or "F-T," is defined as freezing a suspension in liquid nitrogen, and subsequently thawing the suspension in a roughly 30°C water bath.

As used herein, the term "buffer solution" is defined as an aqueous solution or aqueous solution containing less than 25% of a miscible organic solvent, in which a buffer has been added to control the pH of the solution. Examples of suitable buffers include, but are not limited to, PBS (phosphate buffered saline), TRIS (tris-(hydroxymethyl)aminomethane), HEPES (hydroxyethylpiperidine ethane sulfonic acid), and

TES 2-[(tris-hydroxymethyl)methyl]amino-1-ethanesulfonic acid.

As used herein, the term "leaflets" is defined as a single layer of phospholipid in the bilayer forming the liposome.

Selection of Polymerizable Phospholipid

The polymerized liposomes are generally prepared by polymerization of double and/or triple bond-containing monomeric phospholipids. These phospholipids must contain at least polymerizable functional group double bonds, but may contain more than one polymerizable functional group double bond. Examples of polymerizable functional groups include olefins, acetylenes, and thiols. Suitable monomeric phospholipids include DODPC (1,2-di(2,4-Octadecadienoyl)-3-phosphatidylcholine). If the liposome is polymerized by oxidation of thiol groups, it is preferred not to encapsulate thiol-containing biologically active substances, as they could be oxidized during the polymerization step.

Polymerization of Phospholipid

The monomeric double bond-containing phospholipids can be polymerized using a hydrophobic free radical initiator, such as AIBN (azo-bis-isobutyronitrile), or a hydrophilic free radical initiator such as AIPD (azo-bis-amidinopropane dihydrochloride). The latter is particularly useful for initiating polymerization between layers of the bilayer.

The ratio between the phospholipid and crosslinker and aqueous phase affect the percent of crosslinking. In general, the percent crosslinking increases as the amount of crosslinker or time or temperature of reaction are increased. As the percent crosslinking increases, the release rate of the materials from the liposomes decreases and the stability increases.

In a preferred embodiments, the polymerization is carried out at an elevated temperature, from 60 to 100°C, preferably at around 60°C, for 5 to 20 hours, preferably about 5 hours, or until the polymerization is essentially complete. The desired degree of crosslinking is from 30 to 100 percent.

Alternatively, polymerization can be initiated by using a radiation source, such as ultraviolet or gamma radiation. Use of the free radical initiators is preferred if the biologically active substance to be entrapped is denatured when exposed to radiation. Exemplary conditions for initiating polymerization with ultraviolet radiation are to irradiate the solution at 254 nm, 100 W, for three hours at room temperature. Typical conditions for initiating the polymerization with gamma radiation are to irradiate the solution at 0.3 mRad per hour for three hours at room temperature.

Encapsulation of Materials in the Liposomes.

Materials are generally incorporated into the liposomes at the time of formation, following polymerization using sonication of a solution of the material which contains the liposomes, or following polymerization by rehydration of a thin film of the liposomes.

Entrapment prior to Polymerization

The following is a general method for the preparation of polymerized liposomes wherein a biologically active substance is entrapped prior to the polymerization of the monomeric double bond-containing liposome. First, the monomeric liposome is prepared by the thin film hydration of a monomeric double bond-containing phospholipid. The monomeric phospholipid is dissolved, and the solution is then dried to form a thin film of

phospholipid. A solution containing substance to be entrapped is added, preferably with a catalytic amount (1-3 percent by weight) of free radical initiator. It is preferable to establish an inert atmosphere at this stage. The lipid film is then hydrated by gently shaking and sonicating the solution at a temperature of from 30 to 50°C, usually around 40°C, for between five minutes and two hours, preferably around five minutes. Once the lipid film is hydrated, the trap ratio of the liposome can be increased by performing one or more freeze-thaw cycles on the liposome solution. This is particularly useful when the material being incorporated is hydrophilic in nature. The phospholipid components of the liposomes are then polymerized.

Unentrapped biologically active substance can be removed by several means, including repeated centrifugation, decantation, gel filtration, and dialysis. The polymerized liposomes are then suspended in a buffer solution. The buffer solution has a pH preferably between pH 4.5 and pH 9.5, more preferably at physiological pH.

This method of entrapping biologically active substances is preferred because it does not involve the use of organic solvents. Use of organic solvents can denature biologically active substances. Further, the temperature requirements are mild, with the temperature typically not exceeding 60°C.

Entrapment of materials after Polymerization

If the biologically active substance cannot tolerate the temperature conditions or exposure to radiation required for polymerization, the liposomes are polymerized before adding the material to be encapsulated. After the polymerization is complete, the polymerized

liposomes are added to an aqueous solution of the material. The solution should be aqueous, although it can include small amounts of organic solvent. The solution is sonicated to entrap the substance inside the polymerized liposome.

Another method for entrapping biologically active substances in polymerized liposomes is to dissolve the polymerized liposomes in a suitable organic solvent, such as tetrahydrofuran, acetone, ether, chloroform, methylene dichloride, and ethyl acetate, and evaporate the solvent to form a thin film of polymerized liposome. Hydrophobic materials are preferably encapsulated in the liposomes by dissolving the materials in an organic solvent with the phospholipid, before forming the liposomes. Hydrophilic materials are more preferably incorporated by hydrating a thin film of polymerized liposomes in the presence of an aqueous solution of the substance.

Materials can be entrapped within the liposomes, as well as or alternatively in one or more of the lipid layers of the phospholipid bilayer. This is typically determined by the hydrophobicity/hydrophilicity of the material to be incorporated as well as the method of preparation.

Materials to be Incorporated

As used herein, the term "biologically active substance" refers to eukaryotic and procaryotic cells, viruses, proteins, peptides, nucleic acids, polysaccharides and carbohydrates, lipids, glycoproteins, and combinations thereof, and synthetic organic and inorganic drugs exerting a biological effect when administered to an animal. For ease of reference, this term is also used to include detectable compounds such as radiopaque compounds including air and barium, magnetic compounds, fluorescent compounds, and radioactive

compounds. The active substance can be soluble or insoluble in water. Examples of biologically active substances include anti-angiogenesis factors, antiinflammatories, immunomodulators, antibodies, antigens, growth factors, hormones, enzymes, and drugs such as steroids, chemotherapeutics, or antibiotics.

In a diagnostic embodiment, the polymerized liposome incorporates a pharmaceutically acceptable detectable agent, such as a gamma-emitting moiety, like indium or technectium, magnetic particles, radioopaque materials such as air or barium, or fluorescent compounds.

Targeting of Liposomes

The liposomes can be targeted by attachment to the exterior phospholipid moieties of molecules specifically binding to targeted cells or to the mucin lining of the intestine. As used herein, the term "ligand" refers to a ligand attached to the polymerized liposomes which adheres to the mucosa in the intestine or can be used to target the liposomes to a specific cell type in the GI tract or following absorption. These can range from ligands for specific cell surface proteins and antibodies or antibody fragments immunoreactive with specific surface molecules, to less specific targeting such as coatings of materials which are bioadhesive, such as alginate and polyacrylate. In general, ligands are bound to or inserted within the polymerized phospholipids; adhesive polymers are applied as a coating to the particles.

In a further embodiment, magnetic particles can be incorporated into the targeted particles, which are then maintained at the targeted cells using a magnetic field while the particles are imaged or a compound to be delivered is released.

Orally delivered biologically active substances, like food, are generally excreted within two or three days after delivery. However, the polymerized lipospheres can be retained longer if the liposomes are made more "bioadhesive" by attachment of various bioadhesive ligands to the liposomes. These bioadhesive polymerizable liposomes are advantageous over conventional liposomes, and even over polymerized liposomes that lack bioadhesive ligands. Bioadhesive ligands include attachment peptides such as RGD, and extracellular matrix materials such as fibronectin, as well as polymers such as alginate.

Pharmaceutical Compositions and Methods for Administration of the Polymerized Liposomes

The polymerized liposomes are preferably orally administered to a patient, although they can be administered topically or by injection (parenterally). They can also be administered in a capsule. When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier or adjuvant, when the liposomes contain an antigen. If administered topically the liposomes will typically be administered in the form of an ointment or transdermal patch.

The liposomes are preferably administered in an aqueous buffer such as saline or phosphate buffered saline. They are preferably stored under conditions wherein the liposomes remain stable prior to suspension and administration to a patient. The effective dosage is determined by the material which is incorporated into and/or onto the liposomes which is effective when administered orally to achieve the desired effect. This is readily determined by those skilled in the art.

The present invention will be further understood by reference to the following non-limiting examples.

Example 1: Preparation of Polymerized Liposomes.

In a typical preparation, 80 mg of the polymerizable phospholipid, I,2-di(2,4-Octadecadienoyl)-3-phosphatidylcholine (DODPC), 1.68 mg of Azo-bis-isobutyronitrile (AIBN) as a radical initiator, and lipid A derivative as an adjuvant were dissolved in 4 mL chloroform. The solution was dried *in vacuo* to form a thin film on the inside wall of a round bottom flask. A vaccine solution (1 mL of a 1 mg/mL solution) containing 2.68 mg of Azo-bis-(amidinopropane) dihydrochloride (AAPD) as another radical initiator was added to the flask. A nitrogen atmosphere was established and the flask was sealed. The lipid film was gradually hydrated by gently shaking the flask at 40°C for 5 minutes, followed by tip-sonication (10 W for 10 seconds). To increase the trap ratio, the liposome suspension was subjected to three freeze-thaw cycles, by freezing the suspension in liquid nitrogen and then thawing the suspension in a 30°C water bath. The liposome suspension was polymerized at 60°C for 5 hours, at which time polymerization was completed. Polymerization was considered complete when 95 % of the double or triple bonds are crosslinked. Unentrapped vaccine antigen was removed by repeated centrifugation cycles and addition of fresh buffer. Finally, the polymerized liposomes were suspended in the appropriate buffer solution.

The liposomes were in the size range of 4 ± 2 μm diameter as measured by a Coulter Counter particle sizer. The trap ratio of vaccine solution in polymerized liposomes (35%) was similar to that of pre-polymerized liposome preparations. The trap

ratio of 35% is comparable to that of liposomes prepared by other techniques such as the "reverse phase evaporation technique". Further, the water insoluble adjuvant was embedded in the liposome membrane practically without any loss during the preparation process.

The inside structure of each liposome was drastically changed following the freeze-thaw (F-T) treatment, as determined by freeze-fracture electron microscopy. Originally, the liposomes had multi-layered structures and a small volume of inside aqueous phase. The freeze-thaw treatment reduced the number of layers and increased the volume of inner aqueous phase. Additionally, freeze-thaw treatment equilibrated the concentration of solute in the aqueous phase inside and outside of the liposomes. As a result, the trap ratio increased from a few percent to around 35%.

The concentration of entrapped material in the final liposome suspension can be controlled by the original concentration of the material to be entrapped in the aqueous phase used for preparation, and the volume of continuous aqueous phase in which liposomes are suspended.

Example 2: *In vitro* Release of Entrapped Material.

^{14}C labelled bovine serum albumin (BSA) was used as a model protein to follow the *in vitro* release profiles of the material incorporated in liposomes. The polymerized liposomes containing ^{14}C labelled BSA were prepared as described in Example 1. Additionally, non-polymerized liposomes of the same phospholipid composition (DODPC) and of hydrogenated egg phosphatidylcholine and cholesterol (molar ratio 1:1) were prepared as controls by the same procedure but were not

polymerized. The particle sizes and trap ratios of the reference samples were similar to those of the polymerized liposomes.

Release studies were conducted in 1) pH 7.4 isotonic Tris buffered saline, 2) pH 2.0 isotonic HCl saline, and 3) pH 7.4 isotonic Tris saline containing 20 mM sodium taurocholate, 5 U/mL phospholipase A_2, and 2 mM $CaCl_2$. The latter two media simulate physiological conditions in the stomach and the intestine fluid, respectively.

The results of release studies are shown in Figures 1, 2 and 3. Figure 1 displays slow release rates of protein from polymerized liposomes. In acidic solution, less than 5% was released after 6 hours incubation in acidic medium. This would generally be the longest retention time of the liposomes in the stomach, and indicates the polymerized liposomes are stable to acid pH during transit through the stomach. In intestinal-simulated media, there was less than 5% release even after 5 days. These conditions, in general, destabilize liposomes. Figure 2 shows faster release rates from non-polymerized liposomes, of about 90% after 1 day in the intestinal-simulated medium.

The bi-layers in the polymerized liposomes are stabilized by polymerizing the phospholipids. This stabilization is likely the cause of the slower release in the polymerized liposomes relative to the unpolymerized liposomes. Figure 3 shows protein release patterns from hydrogenated egg phosphatidyl choline (PC) liposomes containing cholesterol. Although this liposome preparation is considered the most stable among conventional liposomes, it shows faster release rates in the intestinal-simulated media (about 30% after 5 days) than polymerized liposomes.

Example 3: Measurement of the Absorption of Biologically Active Substances Entrapped in Polymerized Liposomes.

Polymerized liposomes containing ^{125}I-BSA were orally administered to rats. The absorption of ^{125}I-BSA into the blood was then examined. ^{125}I-BSA containing monomeric liposomes and ^{125}I-BSA solution were used as controls. The polymerized liposomes were prepared as described in Example 1. Monomeric liposomes were made of hydrogenated egg phosphatidylcholine and cholesterol (1:1 molar ratio).

Each formulation, including the control ^{125}I-BSA solution, was administered intragastrically with ball-tipped needle and blood was sampled at appropriate intervals from the tail vein. To distinguish between transport of ^{125}I-BSA in the context of liposomes, free ^{125}I-BSA, and the radiolabelled degradation product of ^{125}I-BSA, the blood samples were separated into three fractions: 1) cell debris fraction, 2) trichloroacetic acid (TCA) precipitable fraction, and 3) TCA non-precipitable fraction. Figure 4 shows the sum of the radioactivities belonging to the fractions 1) and 2) in the blood of rats to which each dosage form was administered orally.

The results show that the "effective uptake" is much more from polymerized liposomes administration. Feces of rats were homogenated with water and centrifuged to separate solid stuff. Radioactivity in the whole homogenate and sedimented solid were compared. In the case of polymerized liposome administered rats, almost 80% of total radioactivity was observed in the solid, compared with only 10% from monomeric liposome administered rats. This result suggests that polymerized liposomes are so stable in the GI tract

that the liposomes of this formulation remain as intact structures until excreted.

Because the elimination of the precipitable fraction in blood after intravenous injection was slow, i.e., 75% remaining at 6 hours after injection, the TCA non-precipitable fraction was smaller in animals administered material in polymerized liposomes, as compared to material administered in conventional liposomes and significantly less than when material was administered in solution.

Variations and modifications of the compositions, methods of preparing the compositions, and methods of using the compositions will be obvious to those with ordinary skill in the art. It is intended that all of these variations and modifications be included within the scope of the appended claims.

We claim:

1. A pharmaceutical composition for oral administration to a patient in need thereof comprising liposomes having a diameter of between fifteen nm and ten microns comprising a phospholipid bilayer and an aqueous core having covalently bonded phospholipids in the phospholipid bilayer containing molecules selected from the group of molecules consisting of biologically active drugs, targeting molecules, and detectable molecules, in a pharmaceutically acceptable carrier for oral administration.

2. The composition of claim 1 wherein the targeting molecules are selected from the group consisting of antibodies, antibody fragments, molecules binding to specific cell surface receptors, lectins, and compounds binding to mucin.

3. The composition of claim 1 wherein the detectable molecules are detectable compounds selected from the group consisting of radiopaque substances, radioactive substances, fluorescent substances, air, magnetic materials, and substances detectable by magnetic resonance imaging.

4. The composition of claim 1 wherein the molecules are biologically active substances selected from the group consisting of cells, viruses, proteins, peptides, nucleic acids, polysaccharides and carbohydrates, lipids, glycoproteins, and combinations thereof, and synthetic organic and inorganic drugs exerting a biological effect when administered to an animal.

5. The composition of claim 4 wherein the biologically active substance is an antigen.

6. The composition of claim 1 wherein molecules are incorporated within the hydrophilic layer of the liposome, and molecules are

incorporated within the hydrophobic layer of the liposome.

7. The composition of claim 1 wherein the liposomes have a degree of crosslinking between 30 and 100.

8. The composition of claim 1 wherein the liposomes comprise phospholipids selected from the group consisting of double bond-containing olefinic and acetylenic phospholipids and phospholipids containing thiol groups.

9. The composition of claim 1 wherein the liposomes are prepared by

a) preparing liposomes containing phospholipids selected from the group consisting of double bond-containing olefinic and acetylenic phospholipids and phospholipids containing thiol groups;

b) polymerizing the bonds to covalently link the phospholipids; and

c) entrapping molecules within or into the liposomes.

10. The composition of claim 9 wherein the polymerization is initiated by adding a free radical initiator.

11. The composition of claim 9 wherein the polymerization is initiated by a source of gamma or UV radiation.

12. The composition of claim 9 wherein the phospholipid double bonds are polymerized in a solution of molecules to be incorporated into the liposomes.

13. The method of claim 9 wherein the method further comprises the steps of

dissolving the liposomes in an organic solvent;

evaporating the solvent to form a thin film of polymerized liposomes,

entrapping molecules to be incorporated into the liposomes in the polymerized liposomes by hydrating the thin film of polymerized liposomes in the presence of a solution of the molecules to be incorporated into the liposomes.

14. The composition of claim 13 wherein the liposomes are subjected to one or more freeze-thaw cycles prior to polymerization to entrap material.

15. A method of delivering molecules to an animal by orally administering to an animal liposomes comprising a phospholipid bilayer and an aqueous core having covalently bonded phospholipids in the phospholipid bilayer and molecules selected from the group of molecules consisting of biologically active drugs and detectable molecules.

16. The method of claim 15 further comprising providing in the liposomes targeting molecules selected from the group consisting of antibodies, antibody fragments, molecules binding to specific cell surface receptors, lectins, and compounds binding to mucin.

17. The method of claim 15 wherein the detectable compounds are selected from the group consisting of radiopaque substances, radioactive substances, fluorescent substances, air, magnetic materials, and substances detectable by magnetic resonance imaging.

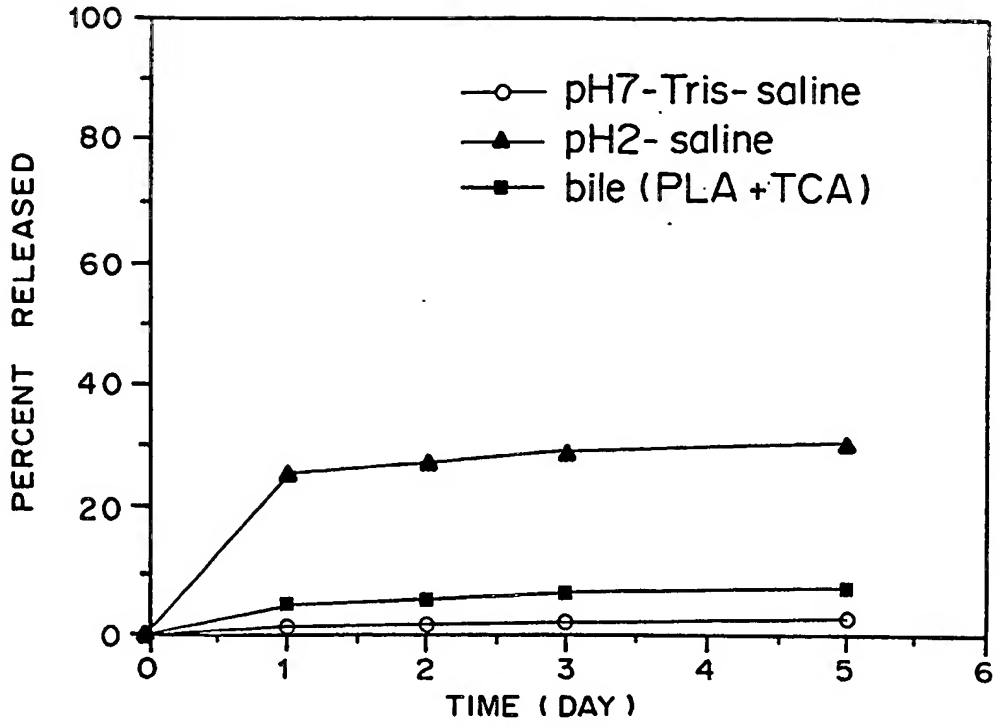
18. The method of claim 17 further comprising detecting the compounds after administration to the animal.

19. The method of claim 15 wherein the molecules are biologically active substances selected from the group consisting of cells, viruses, proteins, peptides, nucleic acids, polysaccharides and carbohydrates, lipids, glycoproteins, and combinations thereof, and

synthetic organic and inorganic drugs exerting a biological effect when administered to an animal.

20. The method of claim 19 wherein the biologically active substance is an antigen and the liposomes are administered to the animal in an amount effective to elicit a humoral or cell mediated immune response against the antigen.

21. The method of claim 20 further comprising providing an adjuvant in the hydrophobic layer of the liposome.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bile (PLA+TCA)
0
1
2
3
4
5
6
TIME (DAY)

FIG. 1

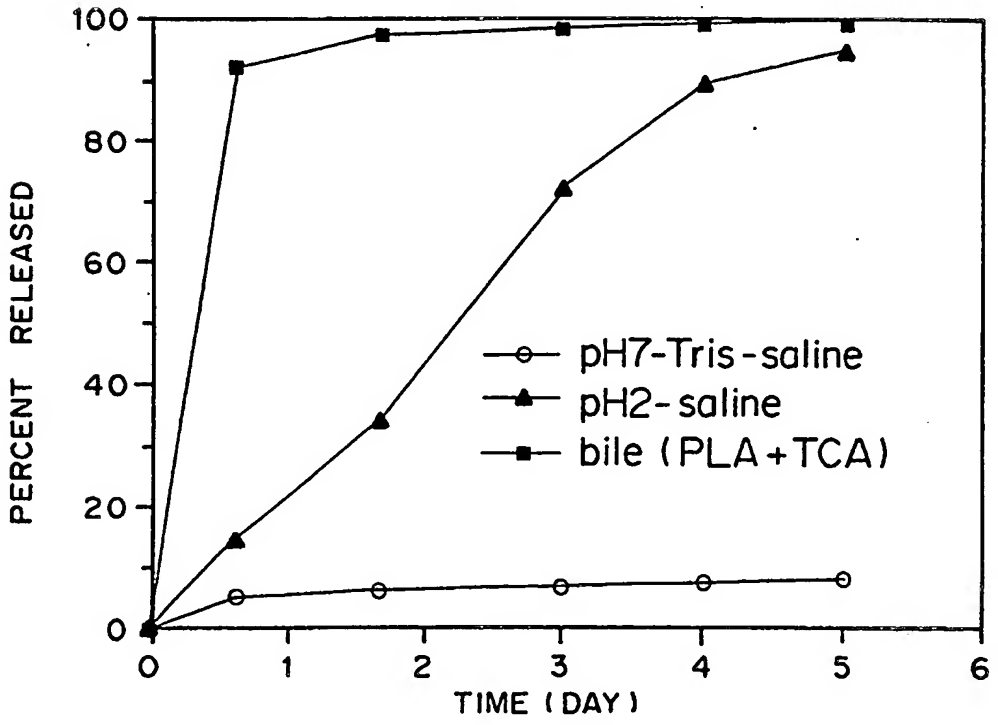
100
80
60
40
20
0
PERCENT RELEASED
pH7-Tris-saline
pH2-saline
bile (PLA+TCA)
0
1
2
3
4
5
6
TIME (DAY)

FIG. 2

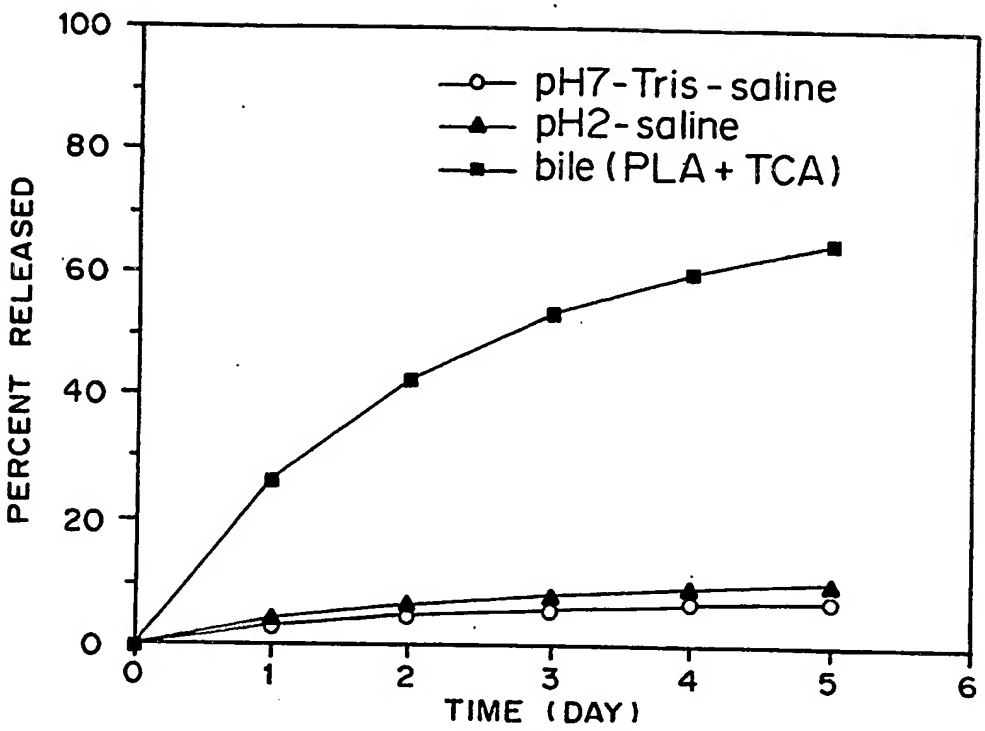
100
80
60
40
20
0
PERCENT RELEASED
pH7-Tris-saline
pH2-saline
bile (PLA + TCA)
0
1
2
3
4
5
6
TIME (DAY)

FIG. 3

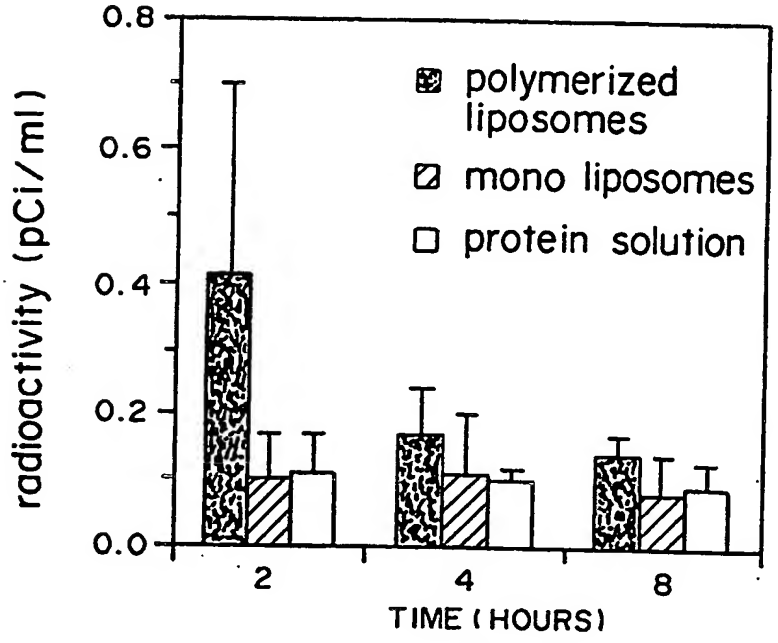
0.8
0.6
0.4
0.2
0.0
radioactivity (pCi/ml)
polymerized liposomes
mono liposomes
protein solution
2
4
8
TIME (HOURS)

FIG. 4

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/US 94/08286

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/127

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CHEMICAL ABSTRACTS, vol. 119, no. 26, 27 December 1993, Columbus, Ohio, US; abstract no. 278618q, J. OKADA ET AL. 'polymerized liposomes for oral drug delivery' page 520 ;column 1 ; see abstract & PROC. INT. SYMP. CONTROLLED RELEASE BIOACT. MATER. 20TH, 1993 pages 302 - 303 ---	1-21
X	DATABASE WPI Week 8525, Derwent Publications Ltd., London, GB; AN 85-149166 & JP,A,60 081 192 (TSUCHIDA) 9 May 1985	1-14
A	see abstract --- -/--	15-21

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 November 1994	17. 11. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Benz, K

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INTERNATIONAL SEARCH REPORT

Internat ' Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	POLYMER NEWS, vol.10, 1984, US pages 68 - 73 S.L. REGEN 'polymerized vesicles' see page 69; table 1	1-14
A	see page 72, paragraph "outlook"	15-21
A	CHEMICAL ABSTRACTS, vol. 118, no. 20, 17 May 1993, Columbus, Ohio, US; abstract no. 197902e, Y.W. CHOI ET AL. 'polymer-coated liposomes for oral drug delivery I' page 407 ;column 1 ; see abstract & YAKCHE HAKHOECHI, vol.22, no.03, 1992 pages 211 - 217	1-21

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